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Cowpea Mosaic Virus

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Introduction

Cowpea mosaic virus (CPMV) is the type member of the genus *Comovirus* which includes 13 additional members in the family *Comoviridae*. CPMV was first isolated from an infected cowpea (*Vigna unguiculata*) plant in Nigeria in 1959. Subsequently, it has been found to occur in Nigeria, Kenya, Tanzania, Japan, Surinam, and Cuba. While its natural host is cowpea, it can infect other legumes, and *Nicotiana benthamiana* has proven to be an extremely valuable experimental host. In nature, CPMV is usually transmitted by leaf-feeding beetles, especially by members of the Chrysomelidae. CPMV has also been reported to be transmitted by thrips and grasshoppers. The beetle vectors can acquire the virus by feeding for as little as one minute and can retain and transmit the virus for a period of days or weeks. The virus does not, however, multiply in the insect vector. Experimentally, CPMV is mechanically transmissible.

In Nigeria, infection of cowpeas with CPMV causes a considerable reduction in leaf area, flower production, and yield. Infected plant cells show a number of characteristic cytological changes. These include the appearance of viral particles, a proliferation of cell membranes and vesicles in the cytoplasm, and a variety of modifications to plasmodesmata.

Physical Properties of Viral Particles

Viral particles can reach a yield of up to 2 g kg^{-1} of fresh cowpea tissue and can be readily purified by polyethylene glycol precipitation and differential centrifugation. The particles are very stable with a thermal inactivation point in plant sap of $65\text{--}75^\circ\text{C}$ and a longevity in sap of 3–5 days at room temperature. Once purified, the particles can be stored for prolonged periods at 4°C . The ease with which virus particles can be propagated, purified, and stored has

undoubtedly contributed to the early popularity of CPMV as an object of study.

CPMV preparations consist of nonenveloped isometric particles, 28 nm in diameter, which can be separated on sucrose density gradients into three components, designated top (T), middle (M), and bottom (B), with sedimentation coefficients of 58S, 95S, and 115S, respectively. The three components have identical protein compositions, containing 60 copies each of a large (L) and small (S) coat protein, with sizes 42 and 24 kDa, respectively, as calculated from the nucleotide sequence. The discovery, in 1971, that CPMV particles contained equimolar amounts of two different polypeptides suggested that the capsids had an architecture more similar to the animal picornaviruses than to other plant viruses of known structure. This provided an early clue as to the common origins of plant and animal viruses.

The difference in the sedimentation behavior of the three centrifugal components of CPMV lies in their RNA contents. Top components are devoid of RNA, while middle and bottom components each contain single molecules of positive-strand RNA of 3.5 and 6.0 kbp, respectively. The two RNA molecules were originally termed middle (M) and bottom (B) component RNA after the component from which they were isolated. However, more recently they have been referred to as RNA-2 and RNA-1, respectively. The three-component nature of CPMV preparations is summarized in [Figure 1](#). The determination of the component structure of the virus, and particularly the relationship between this and infectivity, was important in establishing the principle that plant viruses frequently have divided genomes, the individual components of which are separately encapsidated.

Because of their differing RNA contents, the three components of CPMV also differ in density and can hence be separated by isopycnic centrifugation on cesium chloride gradients. While T and M components give single bands of densities of 1.30 and 1.41 g ml^{-1} , respectively,

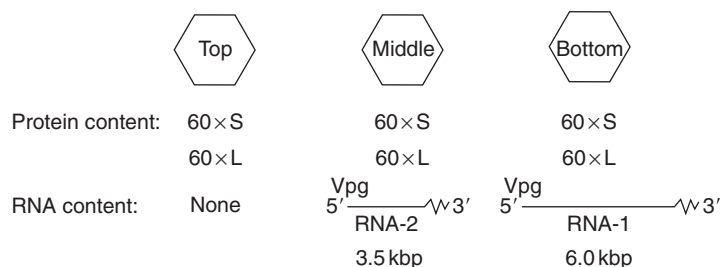


Figure 1 The three-component nature of CPMV indicating the protein and RNA content of each component.

B component gives two bands of 1.43 and 1.47 g ml^{-1} , the proportion of the denser band increasing under alkaline conditions. This increase in density results from an increase in capsid permeability, which allows the exchange of the polyamines present in B components (where they serve to neutralize the excess negative charges from RNA-1) for cesium ions.

CPMV preparations are not only centrifugally heterogeneous but can also be separated in two forms, fast and slow, electrophoretically. Both electrophoretic forms contain all three centrifugal components. The proportion of the two electrophoretic forms in a given virus preparation varies both with the time after infection at which the virus was isolated and the age of the preparation itself. Conversion of one form to the other is caused by loss of 24 amino acids from the C-terminus of the S protein.

Viral Structure

X-ray crystallographic studies on CPMV, as well as the related comoviruses bean pod mottle virus (BPMV) and red clover mottle virus (RCMV), have provided a detailed picture of the arrangement of the two viral coat proteins in the three-dimensional structure of the particle. Overall, the virions are icosahedral, with 12 axes of fivefold and 20 axes of threefold symmetry, and resemble a classic $T=3$ particle. The two coat proteins taken together consist of three distinct β -barrel domains, two being derived from the L and one from the S protein. Thus, in common with the $T=3$ viruses, each CPMV particle is made up of 180 β -barrel structures. The S protein, with its single domain, is found at the fivefold symmetry axes and therefore occupies a position analogous to that of the A-type subunits in $T=3$ particles (Figure 2). The N- and C-termini domains of the L protein occur at the threefold axes and occupy the positions equivalent to those of the C- and B-type subunits of a $T=3$ particle, respectively (Figure 2). This detailed analysis confirmed the earlier suggestion that CPMV particles are structurally homologous to those of picornaviruses, with the N- and C-terminal domains of the L protein being equivalent to viral protein VP2 and viral protein VP3, respectively,

and the S protein being equivalent to viral protein VP1 (Figure 2). However, CPMV particles are structurally less complex than those of picornaviruses. The L and S subunits lack the extended N- and C-termini found in VP2, VP3, and VP1 of picornaviruses and there is no equivalent of VP4. Moreover, CPMV subunits lack the relatively large insertions between the strands of β -sheet, sequences that form the major antigenic determinants of picornaviruses. No RNA is visible in either the M or B components of CPMV, in contrast to the situation found with BPMV where segments of ordered RNA could be detected in middle components.

Genome Structure

Both M and B (but not T) components of a virus preparation are essential for infection of whole plants. As CPMV is a positive-strand RNA virus, a mixture of the genomic RNAs within the particles can also be used to initiate an infection. However, RNA-1 is capable of independent replication in individual plant cells but this leads to the establishment of gene silencing, rather than a productive infection, in the absence of RNA-2. Both genomic RNAs have a small basic protein (VPg) covalently linked to their 5' termini and both are polyadenylated at their 3' ends. The elucidation of the overall structure of the RNA segments once more underscored the similarity between CPMV and picornaviruses. However, unlike picornaviruses, the VPg is linked to the viral RNA via the β -hydroxyl group of its N-terminal serine residue rather than via a tyrosine. The VPg is not required for the viral RNAs to be infectious.

The complete nucleotide sequences of both genomic RNAs were reported in 1983, making CPMV one of the first RNA plant viruses to be completely sequenced. The length of the RNAs are 5889 and 3481 nucleotides, for RNA-1 and RNA-2, respectively, excluding the poly(A) tails and the full sequences appear in GenBank under accession numbers NC_003549 and NC_003550. The two genomic RNAs have no sequence homology apart from that at the 5' and 3' termini. Full-length infectious cDNA clones of both RNAs of CPMV have been

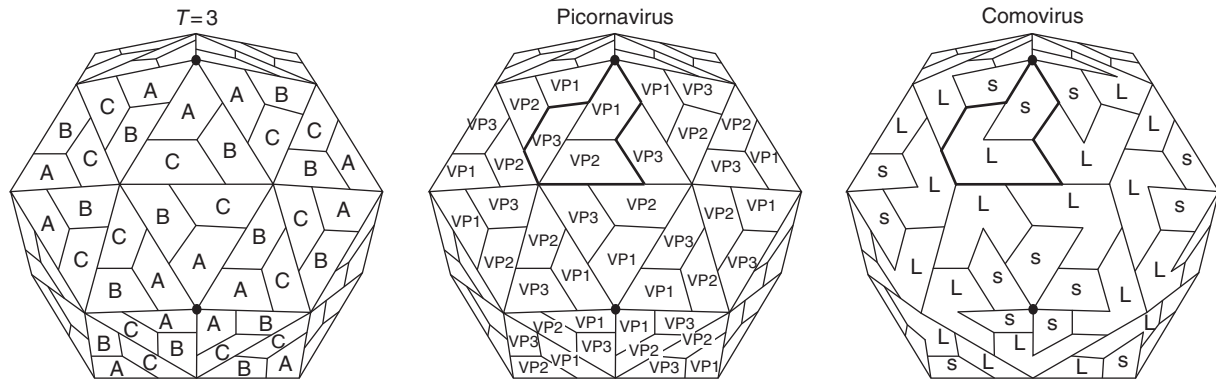


Figure 2 Arrangement of the coat protein subunits of comoviruses (including CPMV) compared with those of simple $T=3$ viruses and picornaviruses. The asymmetric unit of $T=3$ viruses contains three β -barrels contributed by three coat protein subunits with identical amino acid sequences (labeled A, B, and C). The asymmetric unit of a picornavirus also contains three β -barrels, but in this case each is contributed by a different coat protein (labeled viral protein VP1, viral protein VP2, and viral protein VP3). The comovirus capsid is similar to that of a picornavirus except that two of the β -barrels (corresponding to viral protein VP2 and viral protein VP3) are fused to give the L protein. Reproduced from Lomonosoff GP, Shanks M, *et al.* (1991) Comovirus capsid proteins: Synthesis, structure and evolutionary implications. *Proceedings of the Phytochemical Society of Europe* 32: 76.

constructed, allowing the genome to be manipulated. This was an important development since it allowed both reverse genetic experiments to be undertaken and for the virus to be used for biotechnological applications.

Expression of the Viral Genome

Both genomic RNAs contain a single long open reading frame, which occupies over 80% of the length of the RNA. A combination of *in vitro* translation and protoplast studies has unraveled the basic mechanism of gene expression of the virus. Both RNAs of CPMV are expressed through the synthesis and subsequent cleavage of large precursor polypeptides. This was the first example of a plant virus using this strategy for the expression of its genome, a strategy that was subsequently shown to be used by a number of other plant viruses. On RNA-1, initiation of translation occurs at the first AUG encountered on the sequence (at position 207) and results in the synthesis of a protein of approximately 200 kDa (the 200K protein). This initial product undergoes rapid cotranslational autoproteolysis to give proteins with apparent sizes of 32 and 170 kDa (the 32K and 170K proteins). The 170K protein undergoes further cleavages to give the range of virus-specific proteins shown in [Figure 3](#). *In vitro* translation studies using mutant RNA-1 molecules have shown that all the cleavages occur most efficiently *in cis*. The 170K product can initially be cleaved at three different sites to give three different combinations of secondary cleavage products, 58K + 112K, 60K + 110K, and 84K + 87K. *In vitro*, and probably also *in vivo*, the 60K and 110K are stable and do not undergo further cleavage reactions. This is particularly curious in the case of the 110K

protein as it contains both the 24K proteinase domain and a cleavage site. By contrast, the 112K and 84K proteins do undergo further cleavages. The end products of the cleavage pathway of the 170K protein are, from N- to C-terminus, the 58K protein, the VPg, the 24K proteinase, and the 87K protein.

Initiation of translation of RNA-2 occurs at two different positions on the RNA and results in the synthesis of two carboxy coterminal proteins, the 105K and 95K proteins ([Figure 3](#)). This double initiation phenomenon, which occurs as a result of 'leaky scanning', is found with the RNA-2 molecules of all comoviruses. In the case of CPMV, synthesis of the 105K protein is initiated from an AUG at position 161 while initiation from an AUG at position 512 directs the synthesis of the 95K protein. CPMV RNA-2 has an additional AUG (position 115) upstream of both these initiation sites but this feature is not conserved in the RNA-2 molecules of other comoviruses. Both RNA-2-encoded primary translation products are cleaved by the RNA-1-encoded proteolytic activity to give either the 58K or the 48K protein (depending on whether it is the 105K or 95K protein that is being processed) and the two viral coat proteins. Processing of the RNA-2-encoded polypeptides, at least at the site between the 48K and L coat protein, has been shown to require the presence of the 32K protein as well as the 24K proteinase.

Functions of the Viral Proteins

Functions have been ascribed to most of the regions of the polypeptides encoded by both RNA-1 and RNA-2 of CPMV. In most cases, however, it is not certain at what stage(s) in the cleavage pathway they manifest their activity.

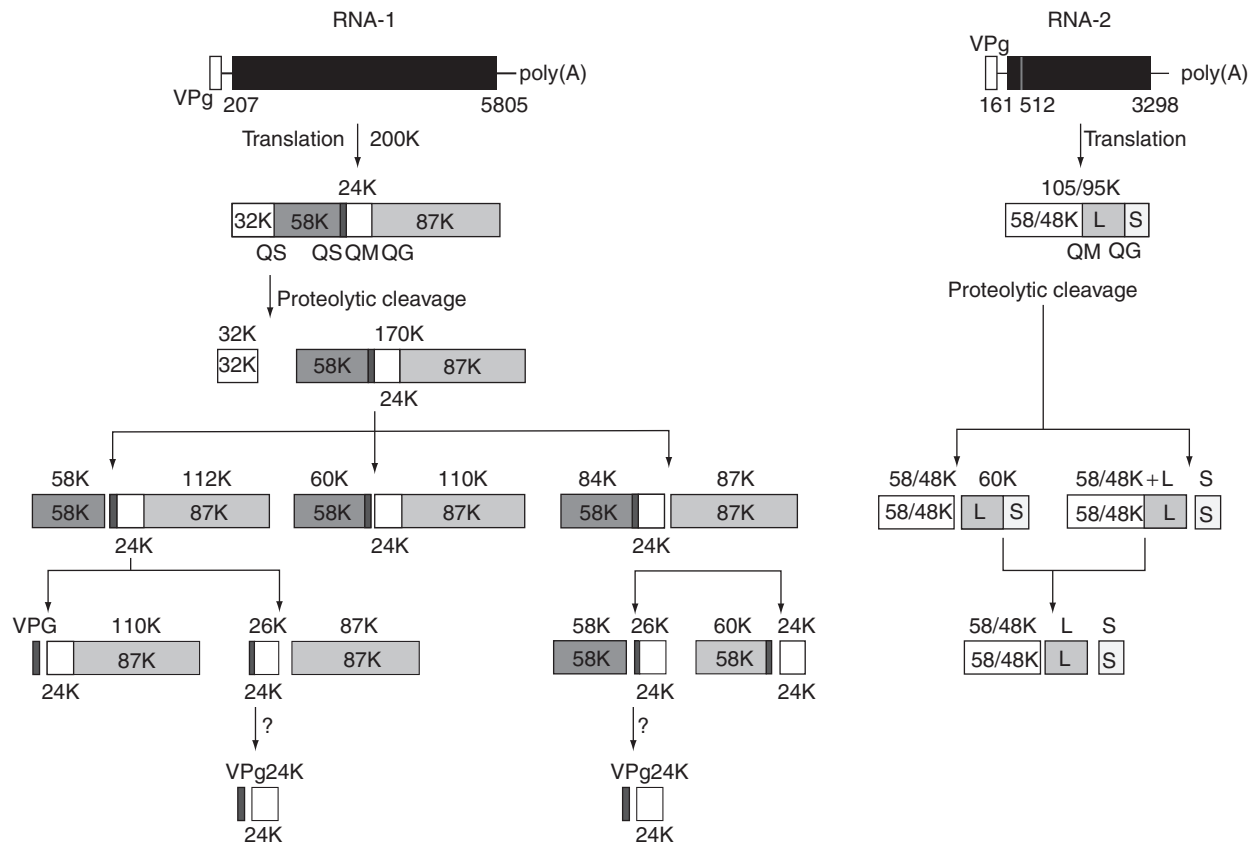


Figure 3 Expression of CPMV RNA-1 and RNA-2. Both RNAs contain a single long open reading frame, which is processed to yield a series of proteins. The positions of the initiation and termination codons and the dipeptides at the proteolytic processing sites are shown.

In the case of RNA-1, the 32K protein, which is rapidly cleaved from the N-terminus of the 200K primary translation product, is a cofactor which modulates the activity of the virus-encoded protease. As described earlier, the presence of the 32K protein is required for the cleavage of the RNA-2-encoded 105K and 95K proteins but is not essential for the cleavage of the RNA-1-encoded 170K protein. It does, however, seem to play a role in determining the rate at which cleavage of the 170K protein occurs. When mutant RNA-1 molecules carrying deletions in the region encoding the 32K protein are translated *in vitro*, the rate of processing of the 170K protein is greatly increased, indicating that the 32K protein acts as an inhibitor of processing. This inhibition may be achieved through the interaction of the 32K with the 58K domain of the 170K protein. The mechanism by which the 32K protein enables the 24K proteinase to cleave *in trans* is unclear.

The RNA-1-encoded 58K protein is associated with cell membranes and contains a nucleotide-binding motif. The 60K protein (Figure 3), containing the amino acid sequence of the 58K protein linked to VPg, is involved

in rearrangements in the endoplasmic reticulum of CPMV-infected cells and acts in concert with the 32K protein. The 24K protein is the virus-encoded proteinase that carries out all the cleavages on both the RNA-1- and RNA-2-encoded polyproteins. Its proteolytic activity has been shown to be expressed in a number of the processing intermediates that contain its sequence. Indeed, it is not known whether the free form of the protein has any biological significance. Although the proteinase contains a cysteine at its active site, it is structurally related to serine proteases, such as trypsin, rather than cellular thiol proteases, such as papain. In this regard, it is similar to the 3C proteinases of picornaviruses. All comoviral cleavage sites identified so far have glutamine (Q) residue at the -1 position. The enzyme encoded by a given comovirus is specific for the polyproteins encoded by that virus and is unable to cleave the polyproteins from other comoviruses either *in cis* or *in trans*.

The 87K protein is believed to contain the virus-encoded RNA-dependent RNA polymerase (RdRp) activity since it contains the G-D-D sequence motif found in all such enzymes. It also has amino acid sequence

homology to the 3D^{Poi} polymerases encoded by picornaviruses. However, when replication complexes capable of elongating nascent RNA chains were isolated from CPMV-infected cowpea plants, they were found to contain the 110K protein (Figure 3), consisting of the sequence of 87K protein linked to the 24K proteinase, rather than the free 87K protein.

In the case of RNA-2, the 48K protein, derived from processing of the 95K protein, is involved in potentiating the spread of the virus from cell to cell. This protein is found in tubular structures that are formed in the plasmodesmata of infected cells. Tubules extending into the culture medium can also be seen in protoplasts either infected with CPMV or transiently expressing the 48K protein. Virus particles can be seen within these tubules when protoplasts are infected with CPMV but not when only 48K protein is expressed. At present, no definite role has been assigned to the 58K protein, which is produced by processing of the 105K protein. Mutants in which translation of the 105K protein is disrupted replicate poorly, if at all. In light of these observations, it has been suggested that the 105K protein may play a role in the replication of RNA-2. Apart from containing many hydrophobic and aromatic amino acids, the approximately 10 kDa of protein present in the 58K but absent from the 48K protein is not conserved between comoviruses. The viral coat proteins are required to enable capsids to be formed. As well as protecting the genomic RNAs, capsid formation is essential for the virus to be able to spread from cell to cell through modified plasmodesmata and long-distance movement also requires capsid formation. An additional function in suppressing gene silencing is also provided by the C-terminal region of the S protein.

Replication

CPMV replicates to high level in infected cells. Replication is believed to involve the initial transcription of the incoming positive-sense RNA into minus-strands followed by initiation and synthesis of new plus-strands from the recently formed minus-strands. It has been shown that the 5' ends of both the plus- and minus-strands are covalently linked to the VPg, suggesting that this protein has an essential role in the initiation of RNA synthesis. There also appears to be a tight linkage between the translation of the viral RNAs and their replication.

Replication of the viral RNAs has been shown to occur in the membraneous cytopathological structures, which are formed in the cytoplasm of cells during infection through the action of the RNA-1-encoded 32 and 60K proteins. Both CPMV-specific double-stranded replicative form (RF) RNA and an enzyme activity capable of completing nascent RNA strands can be isolated from such structures. Purified preparations of the enzyme

activity contain the RNA-1-encoded 110K protein and two host-encoded proteins of 68 and 57 kDa. However, at present, no enzymatic activity capable of initiating RNA synthesis *in vitro* has been described.

Relationships with Other Viruses

Together with the genera *Nepovirus* and *Fabavirus*, the genus *Comovirus* belongs to the family *Comoviridae*. Within the family, the greatest affinity is between the genera *Comovirus* and *Fabavirus*. On a wider scale, consideration of genome structure and organization, translational strategy, and amino acid homologies between the virus-encoded proteins has led to grouping the family *Comoviridae* with the families *Potyviridae* and *Picornaviridae* as members of picorna-like superfamily of viruses. Members of this superfamily are all nonenveloped positive-strand RNA viruses with 3' polydenylated genomic RNAs, which have a protein (VPg) covalently linked to their 5' ends. All members of the supergroup have a similar mode of gene expression, which involves the synthesis of large precursor polypeptides and their subsequent cleavage by a virus-encoded proteinase. The members of the superfamily all contain similar gene order, membrane-bound protein-VPg-proteinase-polymerase (see Figure 3) and share significant amino acid sequence homology in the membrane-bound proteins, the proteinases, and polymerase coding regions. Comovirus capsids are also clearly structurally related to those of picornaviruses (Figure 2).

Use in Biotechnology

CPMV has been extensively used as a vector for the expression of foreign peptides and proteins in plants. To date, all vectors have involved modifications to RNA-2 (Figure 4). In the first instance, antigenic peptides (epitopes) were genetically fused to exposed loops on the surface of the viral capsids. The resulting chimeric virus

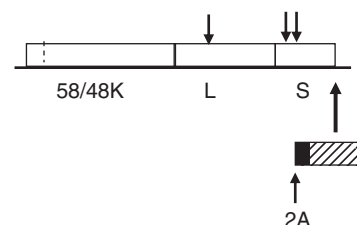


Figure 4 Structure of CPMV RNA-2-based vectors used to express heterologous peptides and proteins in plants. Positions where epitopes have been inserted into the sequence of the coat proteins are shown by black arrows. The position where foreign proteins (shown hatched) have been inserted into RNA-2 is indicated. The FMDV 2A sequence is shown as a black box at the N-terminus of the foreign protein.

particles (CVPs) could be propagated in plants and the modified virions purified. When injected into experimental animals, CVPs can elicit the production of antibodies against the inserted epitope and in a number of instances can confer protective immunity against the pathogen from which the epitope was derived. This was a significant breakthrough as it represented the first instance where protection against an animal pathogen was conferred by material produced from a plant virus-based vector.

In an alternative approach, the sequence encoding an entire heterologous polypeptide has been fused to the C-terminus of the RNA-2-encoded polypeptide via a 2A catalytic peptide derived from foot-and-mouth disease virus (FMDV). The inclusion of the 2A sequence promotes efficient release of the foreign polypeptide from the polypeptide (Figure 4). This system has been used to express antibody derivatives in cowpea plants and crude plant extracts containing the antibodies have been shown to be capable of passively immunizing newborn pigs against challenge with the porcine coronavirus, transmissible gastroenteritis virus.

Recent developments in the use of comoviruses in biotechnology include the creation of combined

transgene/viral vector systems based on CPMV, and the use of CPMV particles in bionanotechnology.

See also: *Nepovirus*; Plant Virus Diseases: Economic Aspects; Poliomyelitis; Virus Particle Structure: Non-enveloped Viruses.

Further Reading

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Cowpox Virus

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History

The first published account of cowpox in man and cattle is probably that of Edward Jenner in his *Inquiry* published in 1798, although others, such as Benjamin Jesty, performed immunization of humans with cowpox material earlier. Jenner described the clinical signs of cowpox in both hosts, and how infection in man with *Variolae vaccinae* ('known by the name of the cowpox') provided protection against smallpox. At that time, smallpox was responsible for between 200 000 and 600 000 deaths each year in Europe and about 10% of all deaths in children. Jenner's discovery, despite the concern of some over the consequences of inoculating bovine material into man, soon led to the establishment of smallpox vaccination schemes around the world. However, not until Pasteur's work c. 100 years later was the principle of immunization used again. In fact, it was Pasteur who suggested that all such immunizations be called vaccines in honor of Jenner's work.

Although Jenner's first vaccines probably came indirectly from cattle, later vaccine material was often derived

from horses, and the origin(s) of modern vaccinia virus (VACV) the smallpox vaccine, remain unknown. That cowpox virus (CPXV) and VACV are different was first published in 1939, since when further biological and genetic studies have confirmed that VACV represents a species in its own right, and is not simply a mutant of CPXV or a recombinant of variola virus (VARV) and CPXV.

Even Jenner seems to have had difficulty finding cowpox cases, and CPXV is not endemic in cattle. Rather, it is endemic in rodents, and cattle and man are merely accidental hosts. The domestic cat is the animal diagnosed most frequently with clinical cowpox in Europe.

Taxonomy and Classification

CPXV represents the species *Cowpox virus*, a member of the genus *Orthopoxvirus* in the family *Poxviridae*, and the international reference strain, Brighton Red, was isolated from farm workers in contact with infected cattle in 1937. CPXV can be differentiated from other orthopoxviruses